Amendments to the Specification:

Please replace paragraph [0026] on page 10 with the following amended paragraph:

[0026] Scrienc *et al.* have reported a flow cytometric method for detecting cloned β-galactosidase activity in the eukaryotic organism, *S. cerevisiae*. The ability of flow cytometry to make measurements on single cells means that individual cells with high levels of expression (*e.g.*, due to gene amplification or higher plasmid copy number) could be detected. In the method reported, a non-fluorescent compound β-naphthol-β-galactopyranoside) is cleaved by β-galactosidase and the liberated naphthol is trapped to form an insoluble fluorescent product. The insolubility of the fluorescent product is of great importance here to prevent its diffusion from the cell. Such diffusion would not only lead to an underestimation of β-galactosidase activity in highly active cells but could also lead to an overestimation of enzyme activity in inactive cells or those with low activity, as they may take up the leaked fluorescent compound, thus reducing the apparent heterogeneity of the population.

Please replace paragraph [0027] on page 10 with the following amended paragraph:

[0027] One group has described the use of a FACS machine in an assay detecting fusion proteins expressed from a specialized transducing bacteriophage in the prokaryote *Bacillus subtilis* (Chung, et.al., J. of Bacteriology, Apr. 1994, p. 1977-1984; Chung, et.al., Biotechnology and Bioengineering, Vol. 47, pp. 234-242 (1995)). This group monitored the expression of a lacZ gene (encodes [[b]]β-galactosidase) fused to the sporulation loci in subtilis (*spo*). The technique used to monitor [[b]]β-galactosidase expression from *spo-lacZ* fusions in single cells involved taking samples from a sporulating culture, staining them with a commercially available fluorogenic substrate for [[b]]β-galactosidase called C8-FDG, and quantitatively analyzing fluorescence in single cells by flow cytometry. In this study, the flow cytometer was used as a detector to screen for the presence of the *spo* gene during the development of the cells. The

device was not used to screen and recover positive cells from a gene expression library or nucleic acid for the purpose of discovery.

Please replace paragraph [0029] on page 11 with the following amended paragraph:

[0029] The lacZ gene from E.coli is often used as a reporter gene in studies of gene expression regulation, such as those to determine promoter efficiency, the effects of *trans*-acting factors, and the effects of other regulatory elements in bacterial, yeast, and animal cells. Using a chromogenic substrate, such as ONPG (*o*-nitrophenyl-[[(]]β-D-galactopyranoside), one can measure expression of β-galactosidase in cell cultures; but it is not possible to monitor expression in individual cells and to analyze the heterogeneity of expression in cell populations. The use of fluorogenic substrates, however, makes it possible to determine β-galactosidase activity in a large number of individual cells by means of flow cytometry. This type of determination can be more informative with regard to the physiology of the cells, since gene expression can be correlated with the stage in the mitotic cycle or the viability under certain conditions. In 1994, Plovins *et al.*, reported the use of fluorescein-Di-β-D-galactopyranoside (FDG) and C_{12}-FDG as substrates for β-galactosidase detection in animal, bacterial, and yeast cells. This study compared the two molecules as substrates for β-galactosidase, and concluded that FDG is a better substrate for β-galactosidase detection by flow cytometry in bacterial cells. The screening performed in this study was for the comparison of the two substrates. The detection capabilities of a FACS machine were employed to perform the study on viable bacterial cells.

Please replace paragraph [0040] on page 14 with the following amended paragraph:

[0040] In another embodiment, the invention provides a method for enriching for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA sample by co-encapsulating a mixture of target DNA obtained from a mixture of organisms

with a mixture of DNA probes including a detectable marker and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity ~~and a detectable marker~~; incubating the co-encapsulated mixture under such conditions and for such time as to allow hybridization of complementary sequences and screening for the target DNA. Optionally the method further comprises transforming host cells with recovered target DNA to produce an expression library of a plurality of clones.

Please replace paragraph [0069] on page 22 with the following amended paragraph:

[0069] The present invention can further optimize methods for isolation of activities of interest from a variety of sources, including one or more consortia[[s]] of microorganisms, primary enrichments, and environmental "uncultivated" samples, to make libraries which have been "normalized" in their representation of the genome populations in the original samples[[.]] and to screen these libraries for enzyme and other bioactivities. Libraries with equivalent representation of genomes from microbes that can differ vastly in abundance in natural populations are generated and screened. This "normalization" approach reduces the redundancy of clones from abundant species and increases the representation of clones from rare species. These normalized libraries allow for greater screening efficiency resulting in the identification of cells encoding novel biological catalysts.

Please replace paragraph [0088] on page 28 with the following amended paragraph:

[0088] After the expression libraries have been generated one can include the additional step of "biopanning" such libraries prior to screening by cell sorting. The "biopanning" procedure refers to a process for identifying clones having a specified biological activity by screening for sequence homology in a library of clones prepared by *(i)* selectively isolating target DNA, from DNA derived from at least one microorganism, by use of at least one probe DNA comprising at least a portion of a DNA sequence encoding [[an]]a biological having the specified

biological activity; and *(ii)* optionally transforming a host with isolated target DNA to produce a library of clones which are screened for the specified biological activity.

Please replace paragraph [0148] on page 42 with the following amended paragraph:

[0148] The abovementioned [[(]]β-galactosidase assays may be employed to screen single *E. coli* cells, expressing recombinant [[(]]β-D-galactosidase isolated from a hyperthermophilic archaeon such as *Sulfolobus solfataricus*, on a fluorescent microscope. Cells are cultivated overnight, centrifuged and washed in deionized water and stained with FDG. To increase enzyme activity, cells are heated to 70°C for 30 minutes and examined with a fluorescence phase contrast microscope. *E. coli* cell suspensions of the [[(]]β-galactosidase expressing clone stained with C_{12}-FDG show a very bright fluorescence inside single cells (Fig 8).

Please replace paragraph [0192] on page 57 with the following amended paragraph:

[0192] Uroporphyrinogen III methyltransferase is an enzyme that catalyzes the S-adenosyl-l-methionine (SAM) -dependent addition of two methyl groups to uroporphyrinogen III methyltransferase to yield dihydrosirohydro-chlorin necessary for the synthesis of siroheme, factor F430 and vitamin B12. The substrate for this enzyme, uroporphyrinogen III (derived from [[ÿ]]δ-aminolevulinic acid) is a ubiquitous compound found not only in these pathways, but also in the pathways for the synthesis of the other so-called "pigments of life", heme and chlorophyll. Dihydrosirohydrochlorin is oxidated in the cell to produce a fluorescent compound sirohydochlorin (Factor II) or modified again by uroporphyrinogen III methyltransferase to produce trimethylpyrrocorphin, another fluorescent compound. These fluorescent compounds fluoresce with a bright red to red-orange color when illuminated with UV light (300nm).

Please replace paragraph [0197] on page 59 with the following amended paragraph:

[0197] In yet another aspect of the present invention, cells expressing molecules of interest are sorted into 96-well or 384-well plates, specifically for further downstream manipulation and screening for recombinant clones. In this aspect of the present invention, ~~the~~ a fluorescence analyzer, such as a FACS machine is employed not to distinguish members of and evaluate populations or to screen as previously published, but to screen and recover positives in a manner that allows further screens to be performed on samples selected. For example, typical stains used for enumeration can affect cell viability, therefore these types of stains were not employed for screening and selecting for further downstream manipulation of cells, specifically for the purpose, for example, of recovering nucleic acid which encodes an activity of interest. In particular, cells containing recombinant clones can be identified and sorted into multi-well plates for further downstream manipulation. There are various ways of screening for the presence of a recombinant clone in a cell. Genes encoding fluorescent proteins, such as green fluorescent protein (Biotechniques 19(4):650-655, 1995), or the gene encoding uroporphyrinogen III methyltransferase (BioTechniques 19:760-764, November 1995) can be utilized in the method of the present invention as reporters to allow detection of recombinant clones. Recombinant clones are sorted for further downstream screening for an activity of interest. Screening may be for an enzyme, for example, or for a small molecule, and may be performed using any variety of methods, including those described or referred to herein.

Please replace paragraph [0211] on page 63 with the following amended paragraph:

[0211] **Phosphorylation of adaptors.** The adaptor ends are phosphorylated by mixing the ligation reaction with 1.0 µl of 10X Ligation Buffer, 2.0 µl of 10mM rATP, 6.0 µl of H_2O, 1.0 µl of polynucleotide kinase (PNK) and incubating at 37°C for 30 minutes. After 30 minutes 31 µl H_2O and 5 ml 10X STE are added to the reaction and the sample is size fractionated on a Sephacryl S-500 spin column. The pooled fractions (1-3) are phenol/chloroform extracted once

followed by an additional chloroform extraction. The DNA is precipitated by the addition of ice cold ethanol on ice for 10 minutes. The precipitate is pelleted by centrifugation in a microfuge at high speed for 30 minutes. The resulting pellet is washed with 1 ml 70% ethanol, repelleted by centrifugation and allowed to dry for 10 minutes. The sample is resuspended in 10.5 µl TE buffer. Do not plate. Instead, ligate directly to lambda arms as above except use 2.5 µl of DNA and no water.

Please replace paragraph [0227] on page 67 with the following amended paragraph:

[0227] To screen for β-galactosidase activity, cells are stained as follows. Cells are cultivated overnight at 37°C in an orbital shaker at 250rpm. Cells are centrifuged to collect about $2x10^7$ cells (0.1ml of the culture), resuspended in 1ml of deionized water, and stained with C_{12}-Fluoroscein-Di- [[(]]β-D-galactopyranoside (FDG). Briefly, 0.5ml of cells are mixed with 50 µl C_{12}-FDG staining solution (1mg C_{12}-FDG in 1ml of a mixture of 98% H_20, 1% DMSO, 1% EtOH) and 50 µl Propidium iodide (PI) staining solution (50 µg/ml of distilled water). The sample is incubated in the dark at 37°C with shaking at 150rpm for 30 minutes. Cells are then heated to 70°C for 30 minutes (this step can be avoided if sample is not derived from a hyperthermophilic organism).

Please replace paragraph [0228] on page 67 with the following amended paragraph:

[0228] The excised λ-ZAP II library is incubated for 2 hours and induced with IPTG. Cells are centrifuged, washed and stained with the desired enzyme substrate, for example C_{12}-Fluoroscein-Di-[[(]]β-D-galactopyranoside (FDG) as in Example 3. Clones are sorted on a commercially available FACS machine, and positives are collected. Cells are lysed according to standard techniques (Current Protocols in Molecular Biology, 1987) and plasmids are transformed into new host by electroporation using standard techniques. Transformed cells are

plated for secondary screening. The procedure is illustrated in Figure 5. Sorted organisms can be grown and plated for secondary screening.

Please replace the Abstract beginning at page 80 with the following amended paragraph:

Disclosed is a process for identifying clones having a specified activity of interest, which process comprises (i) generating one or more expression libraries derived from ~~nuclei~~ nucleic acid directly isolated from the environment; and (ii) screening said libraries utilizing a fluorescence activated cell sorter to identify said clones. More particularly, this is a process for identifying clones having a specified activity of interest by (i) generating one or more expression libraries derived from nucleic acid directly or indirectly isolated from the environment; (ii) exposing said libraries to a particular substrate or substrates of interest; and (iii) screening said exposed libraries utilizing a fluorescence activated cell sorter to identify clones which react with the substrate or substrates. Also provided is a process for identifying clones having a specified activity of interest by (i) generating one or more expression libraries derived from nucleic acid directly or indirectly isolated form the environment; and (ii) screening said exposed libraries utilizing an assay requiring co-encapsulation, a binding event or the covalent modification of a target, and a fluorescence activated cell sorter to identify positive clones.